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Effect of temperature and humidity on the virulence of beetle-derived *Beauveria bassiana*（Balsamo）Vuillemin（Deuteromycetes：Moniliales）against the daikon leaf beetle，*Phaedon brassicae* Baly（Coleoptera：Chrysomelidae）

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Abstract：In this study，the effect of temperature and humidity on the virulence of a *Beauveria bassiana* isolate，SCAU-BB01D，to *Phaedon brassicae*，a serious insect pest of cruciferous vegetables in South China，was studied in the laboratory. The mortality of *P*. *brassicae* was significant lower at temperature of 17℃ and 29℃ than at other temperatures. At temperature of 23℃，the mortality of adult on the 14th day after treatment reached its highest，95.60%. For 2nd instar larvae the highest mortality（96.00%）was obtained at both 23℃ and 26℃ on the 10th day after treatment. The pathogenicity of *B*. *bassiana* to *P*. *brassicae* was enhanced as relative humidity increased. When relative humidity（RH）was increased from 50% to 100%，adult mortality 14 days after treatment was enhanced from 55.70% to 88.80% and 2nd instar larvae mortality 10 days after treatment was enhanced from 62.00% to 96.00%. At 17，20，23，26，and 29℃，the LT_{50} values were 13.63，10.27，8.05，8.87 and 12.41 days for adults，and 6.06，5.72，4.90，4.86 and 6.73 days for 2nd instar larvae，respectively. LT_{50} values at RH of 50%，60%，70%，80%，90% and 100% were 12.97，10.97，10.13，9.79，9.14，8.29 for adults and 7.68，6.92，5.76，5.57，5.48，and 4.82 days for 2nd instar larvae，respectively. Therefore，temperatures between 23 – 26℃ and RH higher than 80% were the most suitable conditions for promoting infection of *B*. *bassiana to P*. *brassicae*.

Key words：*Beauveria bassiana*；*Phaedon brassicae*；temperature；humidity；virulence

1 INTRODUCTION

The daikon leaf beetle，*Phaedon brassicae* Baly（Coleoptera：Chrysomelinae），is an important insect pest in South China（Ge *et al*.，2002）. It frequently damages cruciferous vegetables including cabbage，Chinese flowering cabbage，leaf mustard，radish，kale and watercress and is especially fond of watercress and radish（Li *et al*.，2000）. The beetle can complete five generations within a year and cause very severe damage during May to June and September to November in the region of Guangdong，South China. The current control method for *P*. *brassicae* is through the use of ordinary chemical pesticides. Heavily application of chemical pesticides has caused high resistance to the pesticides by the pest and high concentrations of residues on vegetables. Concerns over pesticide efficacy and safety to the human and the environment have led to the search for alternative management approaches. Biological control experiments with *Bacillus thuringiensis* and entomopathogenic nematodes have been carried out，but the control efficacy of Bt preparations is not stable（Liu *et al*.，1999）and the susceptibility of the larvae of the beetle to nematodes has been variable（Pan *et al*.，1999）.

Beauveria bassiana（Balsamo）Vuillemin（Ascomycota：Hypocreales）is one of the most widely distributed and extensively studied entomopathogenic fungus and is the active agent in many products currently in the use and under development worldwide（Feng *et al*.，1994；Lacey *et al*.，2001）. Because of its attributes of easy mass culture，desiccation stability，compatibility with widely-used formulation ingredients，high activity against a variety of agricultural pests，and essentially no toxicity or infectivity toward vertebrates，it has been used to successfully control many important insect pests such as corn borers，pine caterpillars，Colorado potato beetle，grasshoppers and locusts，*Bemisia*，etc.（Feng *et al*.，1994；Goettel *et al*.，

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1997; Wraight *et al*., 2000). However, until now there has been no published report on the use of *B*. *bassiana* against *P*. *brassicae*.

During the summer of 2001 a number of the beetles with white mycelium were found adhered to stems and leaves of watercress. Field infection rates reached as high as 50% in the watercress-cultivated areas of northern Guangdong. Diseased beetles collected in the field were brought back to the laboratory for the isolation and identification of the pathogen, which was found to be *B*. *bassiana*. After preliminary study of its biology and a comprehensive comparison of 6 field-collected isolates in terms of spore yield, germination rate and pathogenicity against *P*. *brassicae* adults, an isolate, SCAU-BB01D, was selected as the most suitable for further study (He *et al*., 2004). Bioassays of the effect of varying *Beauveria* concentrations on *P*. *brassicae* indicated that this isolate can infest all stages of *P*. *brassicae* in the laboratory with LC_{50} of 1.63×10^5 and 2.68×10^7 conidia/mL to 2nd instar larvae and adults, respectively, and showed high potential for use in microbial control of *P*. *brassicae* (unpublished data).

Environmental conditions, especially temperature and humidity, have long been recognized as key factors influencing the pathogenicity of entomopathogenic fungus and have played a significant role in the incidence of epizootics of insect pathogens (Hegedus and Khachatourians, 1996; Pu and li, 1996; Fargues and Luz, 2000). For example, Daoust and Pereira (1986) demonstrated that temperature and humidity affects both survival and germination rates in *B*. *bassiana*. Sivasankaran *et al*. (1998) showed that at 25℃, the most suitable temperature for infection of *B*. *bassiana* to *Chilo infuscatellus* larvae, the fungal growth, sporulation and mortality of larvae depended mainly on humidity, requiring a high RH of 90% – 100%. Sun *et al*. (2001) reported that mortality of *Helicoverpa armigera* larvae caused by *B*. *bassiana* was the highest at 25℃ and 95% relative humidity in the laboratory. In order to optimize the use of SCAU-BB01D against *P*. *brassicae* in the field, it is necessary to evaluate the effect of temperature and humidity on fungal virulence and find out the most suitable range of temperature and humidity to promote infection of *B*. *bassiana*.

2 MATERIALS AND METHODS

2.1 Test insects

The daikon leaf beetle stock was collected from a field population infesting watercress on a farm managed by the Laboratory of Insect Ecology, South China Agricultural University at Guangzhou, Guangdong Province. The adult beetles were reared on potted watercress plants inside screened cages (25 cm × 25 cm × 40 cm) in the laboratory. After 3 to 5 days of oviposition, *P*. *brassicae* adults were transferred to new potted watercress plants. The watercress plants with eggs were moved to another large screen cage in the laboratory. During the rearing period of more than one month, groups of 2nd instar larvae and adults were selected for use in bioassays.

2.2 Fungal isolates

One collection of *B*. *bassiana* was made from field-collected *P*. *brassicae* at Yangshan County in northern Guangdong Province during the summer of 2001. Daikon leaf beetles showing typical symptoms of *B*. *bassiana* mycosis were collected, taken to the laboratory and used as sources of *B*. *bassiana*. The original isolate A was obtained by isolating *B*. *bassiana* from surface-sterilized beetle cadavers cultivated on PDA petri dishes. Pure cultures of isolates B, C, D, E, and F were obtained by picking up a single mycelium grown on tap water agar and then cultured on PDA media. Among six isolates, isolate D was chosen as the best one based on spore yield, germination rate, and pathogenicity (He *et al*., 2004). Isolate D, SCAU-BB01D, was stored at 4℃ in the Laboratory of Insect Ecology, South China Agricultural University and used for further study.

2.3 Preparation of conidial suspension

Isolate SCAU-BB01D was grown on PDA in Petri dishes (100 mm diameter) in a growth chamber at 25 ± 1℃ and a photoperiod of 12∶12 (L∶D). Conidia on 15-day-old cultures were harvested under sterile conditions by flooding the plate with 10 mL of sterile distilled water and then suspended in 0.01% aqueous (w/v) Tween 80. The suspension was vortexed on a magnetized stirrer for about 30 min to break up the conidial clumps (confirmed under microscopic examination) and then filtered through a piece of double-layer medical gauze to remove the debris. Conidial concentration in the mixture was ascertained with a hemacytometer under a compound microscope and subsequent dilutions were adjusted to 1×10^8 conidia/mL by adding distilled water. The suspensions were used immediately after preparation.

2.4 Bioassay

The larvae and adult beetles were collected from the screened cages in the laboratory just before the bioassay. For each treatment 15 – 20 adult beetles or 20 2nd instar larvae were removed from the host plant and placed in a 90 mm plastic Petri dish. For inoculation, each batch of insects was immersed in a Petri dish with 10 mL of 1×10^8 conidia/mL suspension for 10 s and then moved to a piece of dry filter paper for absorption of extra suspension on the insects. Each treatment consisted of 5 replicates and one control, which was immersed in 0.01% Tween 80 in distilled water. The experiment was conducted with a completely randomized design. After inoculation, treated larvae and beetles were transferred to a plastic Petri dish and fresh greenhouse-grown watercress plants were provided as food. The Petri dishes were covered with a piece of nylon mesh and then transferred to a given growth chambers adjusted to the appropriate temperature and humidity conditions and a 12:12 (L:D) photoperiod. For all the temperature treatments a standard relative humidity of 90% was provided, while a standard temperature of 25℃ was kept for all the humidity treatments.

Treated insects were observed daily for 10 days in the case of larvae and 14 days in the case of adults to record mortality. The watercress plants were replaced daily with fresh ones. All cadavers in all treatments were removed daily and placed in Petri dishes with a damp cloth cover and kept under high humidity to determine the number of sporulating cadavers. Visual observations were also made to record the time until post-mortem mycelial appearance and the time taken to sporulate.

2.5 Temperature and humidity regulation

Five different temperatures of 17℃, 20℃, 23℃, 26℃, and 29℃ were provided using a series of growth incubators and the variation in temperature did not exceed ± 0.5℃. Relative humidities were controlled by using an air pump, three containers connected by rubber tubes, and a Plexiglas experimental chamber placed in a growth incubator with controlled light and temperature, as described by Feng *et al*. (1999). Glycerin and water solutions with particular specific gravities were used to achieve target RH levels of 50%, 60%, 70%, 80%, 90%, and 100% in the Plexiglas chamber (Doberski, 1981).

2.6 Data analysis

Cumulative mortality of all treatments were corrected based on the control group and then analyzed using two-way analysis of variance and Duncan's multiple range test of comparison of means; statistic diffenence of means at $P > 0.05$ was assessed. Mortality data for each treatment were probit-transformed and linearly regressed against incubation time (days) to estimate LT_{50}, confidence limits, and regression parameters using Probit Analysis Procedure in DPS data processing system software (Tang and Feng, 2002).

3 RESULTS

Temperature had a very significant effect on the susceptibility of *P*. *brassicae* to the fungus. The daily cumulative mortality of adults and 2nd instar larvae caused by infection of *B*. *bassiana* under different temperature was showed in Fig. 1. In all temperature treatments the deaths caused by infection of *B*. *bassiana* began to appear on day 6 to 7 for adults (Fig. 1: A) and on day 3 to 4 for larvae (Fig. 1: B), but the peak of mortality differed greatly with temperature. At temperature of 23, 26 and 29℃ the mortality rate of adults peaked between day 7 and 10, and between day 9 and 11 at 20℃; while at 17℃ the peak mortality rate occurred between day 11 and 14. Cumulative mortality at 20 – 26℃ exceeded 80%, while at 17 and 29℃ it did not exceed 60%. For 2nd instar larvae the highest mortality rates occurred between day 3 and 7 in all temperature treatments, but the cumulative mortalities at 23 and 26℃ after 10 days were higher than those of the other three temperature treatments. The daily cumulative mortality of adults and 2nd instar larvae under different humidities after inoculation indicated that high humidity was correlated with higher mortality for both adults and larvae (Fig. 2: A, B).

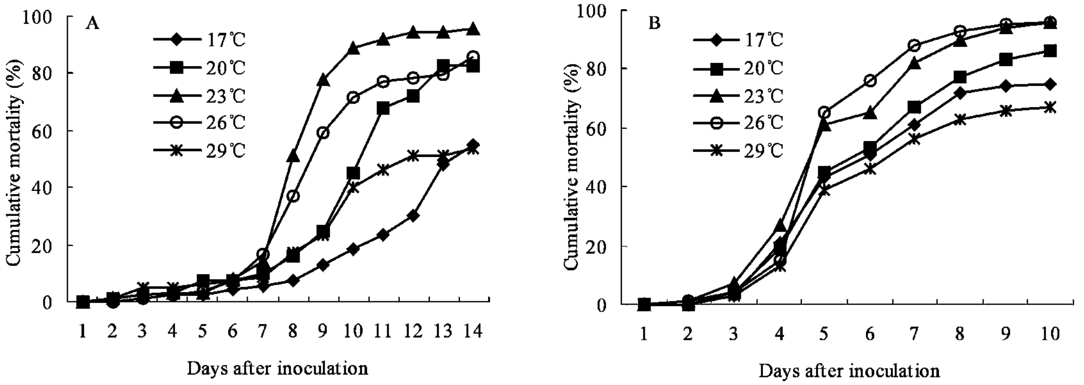

Fig. 1 The daily cumulative mortality of *P*. *brassicae* after treatment with *B*. *bassiana* isolate under different temperature
A: Adult; B: 2nd instar larvae.

The cumulative mortality on day 14 for adults and on day 10 for larvae under different temperature and humidity are shown in Tables 1 and 2. Mean mortality of all treatments was adjusted for the control and then analyzed using two-way analysis of variance and Duncan's multiple range test. At temperatures of 17 and 29℃ the mean mortalities of adults were 54.80% and 53.70% and for 2nd instar larvae 75.00% and

67.00%，respectively，significantly lower than that at other temperatures. The highest adult mortality，95.60%，was obtained at 23℃，while that for 2nd instar larvae（96%）was obtained at 23 and 26℃. Relative humidity had a significant effect on the virulence of *B*. *bassiana* to both adults and larvae. The mortality increased significantly from 55.70% to 88.80% for adults and from 62.00 % to 96.00 % for 2nd instar larvae with humidity of 50% to 100%.

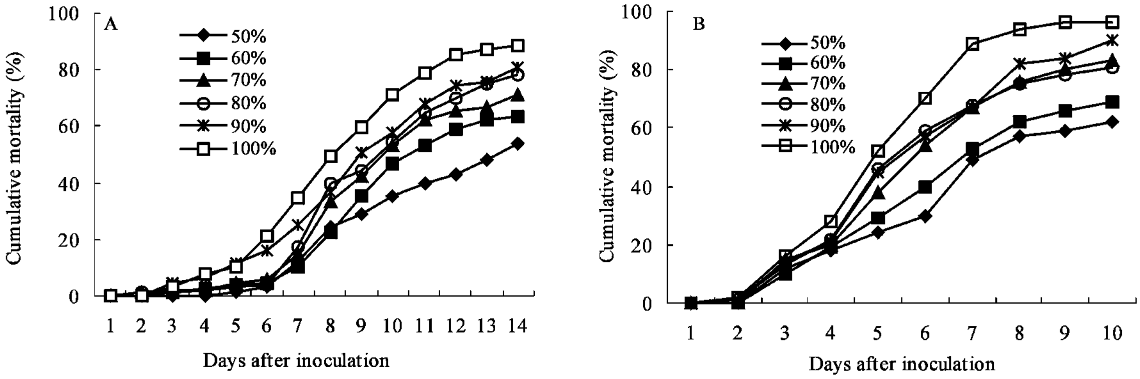

Fig. 2 The daily cumulative mortality of *P*. *brassicae* after treatment with *B*. *bassiana* isolate under different humidity
A: Adult; B: 2nd instar larvae.

Table 1 Comparison of percentage mortality of *P*. *brassicae* caused by *B*. *bassiana* under different temperature for adults on day 14 and for 2nd instar larvae on day 10

Temperature（℃）	Cumulative mortality（%）	
	Adults（on day 14）	2nd instar larvae（on day 10）
17	54.80 ± 1.94 c	75.00 ± 2.24 c
20	82.80 ± 4.30 b	86.00 ± 1.87 b
23	95.60 ± 1.47 a	96.00 ± 1.87 a
26	85.90 ± 1.78 b	96.00 ± 1.00 a
29	53.70 ± 2.01 c	67.00 ± 1.22 d

Notes: Means in the same column followed by the same letter are not significantly different at 5% level（DMRT）. The same for table 2.

Table 2 Comparison of percentage mortality of *P*. *brassicae* caused by *B*. *bassiana* under different humidity for adults on day 14 and for 2nd instar larvae on day 10

Relative humidity（%）	Cumulative mortality（%）	
	Adults（on day 14）	2nd instar larvae（on day 10）
50	55.70 ± 1.59 e	62.00 ± 2.55 c
60	62.20 ± 1.85 d	69.00 ± 4.30 c
70	70.36 ± 1.71 c	83.00 ± 4.06 b
80	78.46 ± 1.46 b	81.00 ± 2.91 b
90	81.10 ± 2.68 b	90.00 ± 3.16 ab
100	88.80 ± 1.37 a	96.00 ± 1.87 a

Regression models of probit-transformed mortality on time were significant difference（$P < 0.0001$）for all temperature and humidity treatments（Tables 3 and 4）.

In the temperature treatments the highest regression slope was obtained at 26℃ with a value of 8.659 for larvae and at 23℃ with value of 13.787 for adults，indicating that the infection rate of *B*. *bassiana* was fastest at these temperatures. As the temperature increased or decreased the slope declined，implying that infection rate of *B*. *bassiana* was slowed down. With the increase of relative humidity from 50% to 100%，the regression slope increased from 3.372 to 6.252 in the assay of 2nd instar larvae，while the highest regression slope（6.097）was reached at humidity of 80% in the assay of adults. Similarly，the shortest LT_{50}，4.86 and 8.05 days，appeared at 26℃ for 2nd instar larvae and at 23℃ for adults，significantly shorter than those in other temperature treatments，suggesting that 23 – 26℃ was the most suitable temperature range for infection of *B*. *bassiana* against *P*. *brassicae*. LT_{50} were curtailed significantly with the enhanced humidity and the shortest LT_{50}，4.82 and 8.29 days for both 2nd instar larvae and adult，were obtained at the highest relative humidity of 100%，indicating that high humidity over 80% was necessary for achieving a higher virulence of *B*. *bassiana* against *P*. *brassicae*.

4 DISCUSSION

Environmental factors such as temperature and humidity are major ones affecting the pathogenicity of entomopathogenic fungi including *B*. *bassiana*. Further，epizootic potential of entomopathogenic fungi have long been considered being mainly dependent upon weather conditions，particularly on moderate temperature and high ambient humidity（Pu and Li，1996）.

Table 3 Regression of probit-transformed mortality on days to death in assays of *B*. *bassiana* against adult and 2nd instar larvae of *P*. *brassicae* under different temperature

Stage	Temperature（℃）	Regression equation（$y = a + bx$）	r	LT_{50}（95% fiducial limits）(d)^a	F^b
2nd instar larva	17	$y = 1.764 + 4.136x$	0.9893	6.06（5.07–7.50）bc	553
	20	$y = 1.203 + 5.012x$	0.9957	5.72（5.17–6.41）b	723
	23	$y = 0.827 + 6.046x$	0.9732	4.90（4.44–5.47）a	1 563
	26	$y = -0.943 + 8.659x$	0.9811	4.86（4.41–5.41）a	653
	29	$y = 1.872 + 3.777x$	0.9724	6.73（5.31–9.11）c	328
Adult	17	$y = -2.603 + 6.701x$	0.9610	13.63（12.51–14.93）d	425
	20	$y = -3.809 + 8.709x$	0.9680	10.27（9.35–11.35）c	1 135
	23	$y = -7.488 + 13.787x$	0.9982	8.05（7.66–8.47）a	913
	26	$y = -1.648 + 7.011x$	0.9912	8.87（7.83–10.19）b	524
	29	$y = 0.169 + 4.416x$	0.9905	12.41（10.35–15.31）d	243

[a] LT_{50} in the same column followed by the same letter are not significantly different at 5% level.

[b] F test showed that all regression equations in the table are significant（$F > F_{0.01}(1, n-2) = 34.12$）. The same for table 4.

Table 4 Regression of probit-transformed mortality on days to death in assays of *B*. *bassiana* against adult and 2nd instar larvae of *P*. *brassicae* under different humidity

Stage	Relative humidity（%）	Regression equation（$y = a + bx$）	r	LT_{50}（95% fiducial limits）(d)^a	F^b
2nd instar larva	50	$y = 2.015 + 3.372x$	0.9740	7.68（6.29–9.76）c	688
	60	$y = 1.912 + 3.676x$	0.9922	6.92（6.27–7.71）c	1 224
	70	$y = 1.594 + 4.481x$	0.9807	5.76（5.20–6.45）b	646
	80	$y = 1.892 + 4.168x$	0.9887	5.57（4.89–6.47）b	1 805
	90	$y = 1.421 + 4.843x$	0.9929	5.48（5.01–6.07）b	2 509
	100	$y = 0.729 + 6.252x$	0.9593	4.82（4.45–5.25）a	4 259
Adult	50	$y = 0.4478 + 4.091x$	0.9914	12.97（11.30–15.10）e	372
	60	$y = 0.263 + 4.554x$	0.9517	10.97（9.85–12.34）d	1 622
	70	$y = -0.265 + 5.236x$	0.9561	10.13（8.80–11.87）cd	1 252
	80	$y = -1.039 + 6.097x$	0.9902	9.79（9.03–10.66）c	802
	90	$y = 0.1579 + 5.037x$	0.9976	9.14（8.45–9.95）b	1 447
	100	$y = 0.1831 + 5.243x$	0.9960	8.29（7.79–8.85）a	1 348

Temperature，in general，influences the germination rate of conidia，hyphal body growth，invasion processes and disease transmission as well as the insect's defenses system（Pu and Li，1996）. Many authors have reported that *B*. *bassiana* germinated and grew most rapidly at 25–30℃（Luz and Fargues，1997，1998；James *et al*.，1998；Sivasankaran *et al*.，1998）. However，the most suitable temperature for infection of *B*. *bassiana* is much lower than this temperature range because of physiological interactions between the host and pathogen. At lower temperatures，conidial germination was greatly delayed and the hyphal body growth rate was greatly inhibited. On the other hand，at higher temperatures the rate of pathogen germination and growth is faster but insects may be able to resist infection because of increased metabolism rate or decreased stadium period leading to more rapid shedding of the cuticle where the fungus attaches and invades（James *et al*.，1998）. The invasion process of *B*. *bassiana* into the insect hemocoele varies with environmental temperatures. In warmer temperatures the fungal invasion is usually accomplished by penetration of germination tubes into insect cuticles，while infection of fungus in low temperatures is mostly attributed to the intestinal invasion caused by germination of conidia ingested by the insect（Lin *et al*.，1998）. Therefore， mycosis and mortality of insects in the laboratory and in the field would be expected to decrease as temperatures increase to the optimum temperature range for fungal growth and sporulation (Inglis *et al*., 1997; James *et al*., 1998).

The results presented here support the conclusions of Inglis *et al*. (1997) and James *et al*. (1998). In our previous paper, the effect of temperatures on biological characteristics of *B*. *bassiana* isolate SCAU-BB01D *in vitro* was studied and the results showed that 26℃ was optimum temperature for sporulation, conidial germination and mycelia growth (Kuang *et al*., 2005). In the present study, temperature 23℃ and 26℃ were the most suitable temperature for *B*. *bassiana* infecting adults and 2nd instar larvae of *P*. *brassicae*, respectively, with the highest cumulative mortalities (95.60% for adults at 23℃, 96% for 2nd instar larvae at 23℃ and 26℃), the sharpest regression slopes (13.787 for adults at 23℃ and 8.659 for 2nd instar larvae at 26℃) and the shortest LT_{50} (8.05 days for adult at 23℃ and 4.86 days for 2nd instar larvae 26℃).

Numerous studies clearly showed that conidial germination of *B*. *bassiana*, its virulence to different species of insects and sporulation on fungus-killed cadavers were highly dependent on the moisture conditions (Doberski, 1981; Daoust and Pereira, 1986; Luz and Fargues, 1997; Luz *et al*., 1999; Fargues and Luz, 1998, 2000). A high ambient humidity is essential in inducing fungal epizootics in insect populations in the field (Pu and Li, 1996). For example, Fargues and Luz (2000) reported that daily high humidity (nearly saturated) was the most crucial climatic constraint for control of *Rhodnius prolixus*, a vector of Chagas' disease, by using of *B*. *bassiana*. James *et al*.(1998) demonstrated that the highest field mortality of a coccinellid, *Hippodamia convergens*, occurred at low temperature (≤20℃) following *B*. *bassiana* applications when humidity level was above 96% for at least 24 h. Our research results indicated that humidity had a significantly effect on the virulence of *B*. *bassiana* to both adults and larvae of *P*. *brassicae*. When relative humidity increased from 50% to 100%, the cumulative mortalities of adult and larva were enhanced, the slopes of regression model rose, but LT_{50} were shortened, coincident with the results of other authors. Although much higher humidity is required for conidial germination, *B*. *bassiana* infections have been shown to occur at humidity as low as 50% with 55.70% and 62.00% mortality for adults and 2nd instar larvae, respectively, implying that the tested *B*. *bassiana* isolate (SCAU-BB01D) has some tolerance to low humidity.

The larvae of *P*. *brassicae* are more susceptible to infection of *B*. *bassiana* than the adults, and this was shown in lower mortalities and shorter LT_{50} of 2nd instar larvae than those of the adults in all treatments of temperature and humidity. At the concentration of 1×10^8 conidia/mL, the median lethal times (LT_{50}) for adults was 8 – 13 days, distinctly longer than the LT_{50} for larvae (5 – 8 days). The differences in susceptibility may be due to variation in morphological characteristics. Usually, Coleopteran adults are less susceptible to fungal infection than larvae due to their special elytra and sclerotized structure, although similar results have been shown in several Coleopterous insects such as *Henosepilachna vigintioctopunctata* (Padmaja *et al*., 1998) and the Colorado potato beetle, *Leptinotarsa decemlineata* (Jaros-Su *et al*., 1999).

Based on the bioassay data in the laboratory, we concluded that a temperature range of 23 – 26℃ and relative humidity over 80% were the most suitable for *B*. *bassiana* infecting *P*. *brassicae*. Apart from temperature and humidity, the virulence of *B*. *bassiana* and its actual performance in the field is also influenced by other factors such as concentration of conidia suspension, application techniques, insect densities, etc. In addition, the interaction between temperature and humidity will affect the virulence to some extent. Therefore, future research will focus on the evaluation of pathogenicity and control efficacy of this isolate against *P*. *brassicae* in the field.

Watercress is a major cruciferous vegetable for Cantonese dishes in South China, but grows better at mild climate. The summer temperatures in the area of Pearl River Delta are too high for farmers to grow watercress. Therefore in recent years large-scale cultivation of watercress have shifted to mountainous areas in northern Guangdong and watercress is planted in valley fields near streams or below reservoirs, where the climate is mild and there is sufficient running water for watercress production. In mountainous areas *P*. *brassicae* occurs frequently and results in severe damage due to year-round watercress cultivation (Wang *et al*., 1993). However, the particular micro-environmental conditions in watercress cultivation, such as a moderate temperature and high humidity, are nearly optimum conditions for infection of *B*. *bassiana* against *P*. *brassicae*. Thus, *B*. *bassiana* isolate SCAU-BB01D has a high potential for microbial control of *P*. *brassicae* in the watercress cultivation, especially in mountainous region of Guangdong Province.

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References

Daoust RA, Pereira RM, 1986. Survival of *Beauveria bassiana*

（Deuteromycetes：Moniliales）conidia on cadavers of cowpea pests stored outdoors and in the laboratory in Brazil. *Environ. Entomol.*，15：642–647.

Doberski JW，1981. Comparative studies glycerol solution for humidity control. *J. Invertebr. Pathol.*，37：195–200.

Fargues J，Luz C，2000. Effects of fluctuating moisture and temperature regimes on the infection potential of *Beauveria bassiana* for *Rhodnius prolixus*. *J. Invertebr. Pathol.*，75：202–211.

Fargues J，Luz C，1998. Effects of fluctuating moisture and temperature regimes on sporulation of *Beauveria bassiana* on cadavers of *Rhodnius prolixus*. *Biocontrol Sci. Technol.*，8：323–334.

Feng MG，Poprawski TJ，Khachatourians GG，1994. Production，formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control：Current status. *Biocontr. Sci. Technol.*，4：3–34.

Feng MG，Xu Q，Xu JH，1999. Humidity control in biological experiments：modified device and methodology. *Chin. J. Appl. Ecol.*，10（3）：357–361.

Ge SQ，Wang SY，Yang XK，2002. Notes on the genus *Phaedon* Latreille of China（Coleoptera：Chrysomelidae：Chrysomelinae）. *Acta Zootaxonomica Sinica*，27（2）：316–325.［葛斯琴，王书永，杨星科，2002. 中国猿叶甲属种类记述. 动物分类学报，27（2）：316–325］

Goettel MS，Jaronski ST，Prior C，1997. Safety and registration of microbial agents for control of grasshoppers and locusts. *Men. Entomol. Soc. Can.*，171：83–99.

He YR，Lu LH，Kuang ZB，Liu XM，Zeng HM，2004. Biological aspects and pathogenicity of beetle-derived *Beauveria bassiana* isolates to *Phaedon brassicae*. *Entomol. Knowledge*，41（5）：442–445.［何余容，吕利华，邝灼彬，刘学明，曾惠明，2004. 球孢白僵菌不同分离株的生物学及对小猿叶甲成虫致病性测定. 昆虫知识，41（5）：442–445］

Hegedus DD，Khachatourians GG，1996. The effects of temperature on the pathogenicity of heat-sensitive mutants of the entomopathogenic fungus，*Beauveria bassiana*，toward the migratory grasshopper，*Melanoplus sanguinipes*. *J. Invertebr. Pathol.*，68：160–165.

Inglis GD，Johnson DL，Cheng KJ，1997. Use of pathogen combinations to overcome the constraints of temperature on entomopathogenic hyphomycetes against grasshoppers. *Biol. Contr.*，8：143–152.

James RR，Croft BA，Shaffer BT，Lighthart B，1998. Impact of temperature and humidity on host-pathogen interactions between *Beauveria bassiana* and a coccinellid. *Environ. Emtomol.*，27（6）：1 506–1 513.

Jaros-Su J，Groden E，Zhang JX，1999. Effect of selected fungicides and the timing of fungicide application on *Beauveria bassiana*-induced mortality of the Colorado potato beetle（Coleoptera：Chrysomelidae）. *Biol. Contr.*，15：259–269.

Kuang ZB，Lu LH，Feng X，Chen HY，Wu YJ，He YR，2005. Effect of temperature and chemical pesticides on the biological characteristics of *Beauveria bassiana*. *J. South China Agri. Uni.*，26（3）：26–29.［邝灼彬，吕利华，冯夏，陈焕瑜，武亚静，何余容，2005. 温度和常见农药对球孢白僵菌菌株生物学特性的影响. 华南农业大学学报，26（3）：26–29］

Lacey LA，Frutos R，Kaya HK，Vail P，2001. Insect pathogens as biological control agents：Do they have a future？*Bio. Contr.*，21：230–248.

Li WF，Gu DJ，Chen YG，Chen ZP，2000. Studies on effects of vegetable varieties on biology of *Phaedon brassicae* Baly. *J. South China Agri. Uni.*，21（2）：38–40.［李伟丰，古德就，陈亦根，陈志平，2000. 蔬菜品种对小猿叶甲生物学特性影响的研究. 华南农业大学学报，21（2）：38–40］

Lin HF，Fan MZ，Li ZZ，Hu C，1998. Pathogenic effect of *Beauveria bassiana* infected on *Dendrolimus punctus* under different temperature and humidity. *Chinese J. Appl. Ecol.*，9（2）：195–200.［林华峰，樊美珍，李增智，胡萃，1998. 不同温湿度下白僵菌对松毛虫的侵染致病效应. 应用生态学报，9（2）：195–200］

Liu M，Sun M，Yu Z，1999. Bioassay to assess the toxicity of *Bacillus thuringiensis* to Daikon leaf beetle（Coleoptera：Chrysomelidae）. *J. Invertebr. Pathol.*，74：255–260.

Luz C，Fargues J，1997. Temperature and moisture requirements for conidial germination of an isolate of *Beauveria bassiana*，pathogenic to *Rhodnius prolixus*. *Mycopathologia*，138（3）：117–125.

Luz C，Fargues J，1998. Factors affecting conidial production of *Beauveria bassiana* from fungus-killed cadavers of *Rhodnius prolixus*. *J. Invertebr. Pathol.*，72（2）：97–103.

Luz C，Silva IG，Cordeiro CM，Tigano MS，1999. Sporulation of *Beauveria bassiana* on cadavers of *Triatoma infestans* after infection at different temperatures and doses of inoculum. *J. Invertebr. Pathol.*，73（2）：223–225.

Padmaja V，Gurvinder K，Kaur G，1998. Relative susceptibility of brinjal spotted beetle，*Henosepilachna vigintioctopunctata*（Fabricius）to certain isolates of *Beauveria bassiana*（Bals.）Vuill. *J. Biol. Contr.*，12：147–151.

Pan KX，Lu XJ，Liu NX，Liu XL，Li QJ，1999. Test on the sensitivity of *Phaedon brassicae* to *Steinernema carpocapsae* A24. *Natural Enemies of Insects*，21（2）：93–95.［潘克鑫，陆小军，刘南欣，刘秀玲，李秋剑，1999. 小卷蛾线虫对小猿叶甲幼虫的敏感性实验初报. 昆虫天敌，21（2）：93–95］

Sivasankaran P，Easwaramoorthy S，David H，1998. Influence of temperature and relative humidity on the growth，sporulation and pathogenicity of *Beauveria* nr. *bassiana*. *J. Biol. Contr.*，12（1）：71–76.

Sun LJ，Wu KM，Guo YY，2001. The pathogenicity of *Beauveria bassiana* to *Helicoverpa armigeria* under different temperatures and humidities. *Acta Entomol. Sinica*，44（4）：501–506.［孙鲁娟，吴孔明，郭予元，2001. 不同温、湿度下僵菌对棉铃虫幼虫的致病力. 昆虫学报，44（4）：501–506］

Tang QY，Feng MG，2002. DPS Data Processing System for Practical Statistics. Beijing：Scientific Press.［唐启义，冯明光，2002. 实用统计分析及其 DPS 数据处理系统. 北京：科学出版社］

Wang WB，Xia LD，1993. Biological characteristics and chemical control of *Phaedon brassicae* Baly. *J. Changjiang Vegetables*，1：21.［汪万宝，夏理定，1993. 小猿叶甲习性特点及药剂防治实验. 长江蔬菜，1：21］

Wraight SP，Carruthers RI，Jaronski ST，Bradley CJ，Garza CJ，Galaini-Wraight S，2000. Evaluation of the entomopathogenic fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for microbial control of the silverleaf whitefly，*Bemisia argentifolii*. *Biol. Contr.*，17：203–217.

Pu ZL，Li ZZ，1996. Insect Mycology. Hefei：Anhui Publishing House of Science and Technology. 112–140.［蒲蛰龙，李增智，1996. 昆虫真菌学. 合肥：安徽科学技术出版社. 112–140］

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不同温湿度下球孢白僵菌对小猿叶甲的致病力

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摘要：本文在室内研究了分离自小猿叶甲的一株球孢白僵菌（SCAU-BB01D）在不同温度和湿度条件下对小猿叶甲成虫和 2 龄幼虫的致病力。结果显示温度和相对湿度对白僵菌的致病力有显著影响。在 17℃和 29℃，小猿叶甲成虫和 2 龄幼虫的死亡率显著低于其他温度；在 23℃下，成虫接菌后第 14 天的累计死亡率达到最高为 95.60%；在 23℃和 26℃下，2 龄幼虫接菌后第 10 天达到最高累计死亡率 96%。随着相对湿度的提高，球孢白僵菌对小猿叶甲的致病力显著增加，当相对湿度从 50%增加至 100%时，成虫第 14 天的累计死亡率从 55.70%增加到 88.80%，2 龄幼虫第 10 天的累计死亡率从 62.00%增加到 96.00%。机率值分析结果表明，在 17、20、23、26 和 29℃温度下，白僵菌对小猿叶甲成虫和 2 龄幼虫的 LT_{50} 分别为 13.63、10.27、8.05、8.87、12.41 天和 6.06、5.72、4.90、4.86、6.73 天。随着相对湿度的升高，球孢白僵菌对小猿叶甲的致死中时（LT_{50}）呈缩短的趋势，当相对湿度为 50%、60%、70%、80%、90%、100%时，成虫和 2 龄幼虫的 LT_{50} 分别为 12.97、10.97、10.13、9.79、9.14、8.29 天和 7.68、6.92、5.76、5.57、5.48、4.82 天。由实验结果可以得知，23～26℃是球孢白僵菌感染小猿叶甲最适宜的温度范围，环境湿度越高越有利于病原菌侵染，当相对湿度大于 90%时，最有利于球孢白僵菌对小猿叶甲的感染。

关键词：球孢白僵菌；小猿叶甲；温度；湿度；致病力

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